INFECTION AND IMMUNITY, Oct. 1975, p. 728-732

Vol. 12, No. 14
Printed in U.S.A.

# Type Specificity of Complement-Requiring and Immunoglobulin M Neutralizing Antibody in Initial Herpes Simplex Virus Infections of Humans

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Received for publication 7 May 1975

Studies comparing the enhancing effect of guinea pig complement on homotypic and heterotypic neutralizing antibodies produced in initial herpes simplex virus (HSV) infections of humans indicated that antibodies to HSV type 1 and HSV type 2 were enhanced to about the same extent, and there was no significant difference in the degree to which complement enhanced homotypic and heterotypic HSV-neutralizing antibody. Homotypic and heterotypic immunoglobulin G neutralizing antibodies were enhanced by complement to as great, or greater, an extent as immunoglobulin M (IgM) HSV antibodies in the same sera. In patients with initial HSV type 1 infections, the IgM neutralizing antibody response was type specific. On the other hand, patients with initial HSV type 2 infections produced both homotypic and heterotypic IgM neutralizing antibody. An initial HSV type 2 infection in an individual previously infected with HSV type 1 elicited the production of IgM neutralizing antibody to both HSV type 1 and HSV type 2. However, patients with recurrent HSV type 1 infections failed to produce IgM antibody to either HSV type during reactivation of the virus.

Various investigators have demonstrated enhancement of herpes simplex virus (HSV)-neutralizing antibody by fresh guinea pig complement (5, 6, 8, 10, 12, 13), but information is lacking on the type specificity of complement-requiring neutralizing antibodies in HSV infections of humans, and on the degree to which complement enhances neutralizing antibody in various classes of immunoglobulins in human HSV infections. However, in experimental infections of rabbits, Hampar et al. (5) found that antibodies in different classes of immunoglobulins varied in their complement requirements for neutralization of HSV.

It is well established that immunoglobulin M (IgM) antibody is produced in initial infections with HSV, but little has been reported on the type specificity of the response. Hampar et al. (4) reported that, in rabbits immunized with HSV types 1 and 2, the late 19S neutralizing antibodies were highly type specific, whereas early 19S and both early and late 7S antibodies lacked type specificity. Investigators using the indirect hemagglutination (IHA) test for assay of HSV antibody have detected type-specific IgM antibody in early sera from human type 2 HSV infections (1, 9; W. A. Farris, J. A. Stewart, and J. R. Evrard, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, p. 256, V 337).

The present report describes the results of studies on the type specificity of the IgM-neutralizing antibody produced in initial HSV type 1 and type 2 infections, and the enhancing effect of complement on homotypic and heterotypic IgM and immunoglobulin G (IgG) neutralizing antibodies elicited by initial HSV infections. The use of a highly sensitive and specific radioimmunoassay method (B. Forghani, N. J. Schmidt, and E. H. Lennette, J. Clin. Microbiol., in press) for typing HSV antibody permitted accurate determination of the patients' current infecting virus type and indicated whether the patients had previously experienced an infection with the HSV heterotype.

## MATERIALS AND METHODS

**Neutralization tests.** The MacIntyre strain of HSV type 1 (HSV-1), obtained from the American Type Culture Collection, and the Johnson strain of HSV type 2 (HSV-2) were employed for neutralizing antibody assays. The latter strain was isolated in this laboratory from a neonatal herpetic infection and was typed by A. Nahmias.

The plaque reduction neutralization test for HSV antibodies was performed as described elsewhere (11) in human fetal diploid lung cells in Linbro FB-6 plates (Linbro Chemical Co., New Haven, Conn.). Hanks balanced salt solution containing 5% inactivated fetal bovine serum was used as a diluent for serum, virus, and guinea pig complement. Tests were conducted in parallel in the absence of fresh guinea pig complement, and in the presence of ap-

proximately 10 hemolytic units of complement in the serum-virus mixtures as described by Benyesh-Melnick (2) for neutralization tests for human cytomegalovirus. Preliminary experiments indicated that identical neutralizing antibody titers were obtained in the absence of guinea pig serum and in the presence of heated guinea pig serum. Each serum-virus mixture was inoculated in duplicate into two cell culture dishes. HSV-neutralizing antibody titers were expressed as the highest dilution of serum or gradient fraction producing a 50% or greater reduction in plaque count.

**Separation of IgM and IgG antibodies.** Immunoglobulins were separated by sucrose density gradient centrifugation as described elsewhere (3). The 19S (IgM) and 7S (IgG) fractions were dialyzed against physiological saline for 18 h at 4 C, and then each was concentrated to a volume representing a 1:2 dilution of whole serum by dialysis against Aquacide I (Calbiochem). The specificity and purity of the immunoglobulin content of each fraction was tested by double immunodiffusion tests (3) against gamma-chain-specific anti-human IgG and mu-chain-specific anti-human IgM from Behring Diagnostics.

**Typing of HSV antibodies.** The type-specific identification of HSV antibodies by a solid-phase radioimmunoassay is described elsewhere (B. Forghani, N. J. Schmidt, and E. H. Lennette, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, p. 256, V 333; J. Clin. Microbiol., in press). It is based upon absorbing sera with cells infected with HSV-1 or HSV-2 and testing for residual antibody.

**Human sera examined.** Paired sera were selected from 15 patients who were apparently experiencing an initial infection with HSV. In addition to clinical findings, the diagnosis of an initial HSV infection was based upon the absence of complement-fixing antibody to HSV in the acute-phase serum specimen and upon subsequent seroconversion. That the HSV infections in these patients represented initial ones was further substantiated by the demonstration of HSV IgM neutralizing antibodies in their sera, and by the fact that antibody typing by radioimmunoassay indicated antibody to only a single HSV type. HSV-1 was isolated from four of seven patients with HSV-1 antibodies, and HSV-2 was isolated from three of the eight patients with HSV-2 antibodies. Sera were also selected from six patients with a history of recurrent HSV infections; typing by radioimmunoassay revealed only HSV-1 antibody in their sera.

## RESULTS

**Enhancement of homotypic and heterotypic HSV-neutralizing antibodies by complement.** Figure 1 compares acute and convalescent titers of homotypic and heterotypic HSV-neutralizing antibody for the 15 patients with initial HSV infections. The acute-phase sera from HSV-1 patients were collected from 1 to 4 days after onset of infection, and only two had low levels of homotypic neutralizing antibody, demonstrable only in the presence of complement. Acute-phase sera from the HSV-2 patients were collected from 1 to 9 days after onset, and a few of the later ones had low levels of homotypic and heterotypic neutralizing antibody, particularly in the presence of complement. The type 1 patients tended to have higher homotypic titers than heterotypic titers, but the type 2 patients had levels of type 1 antibody as high or higher than those to homotypic type 2 virus.

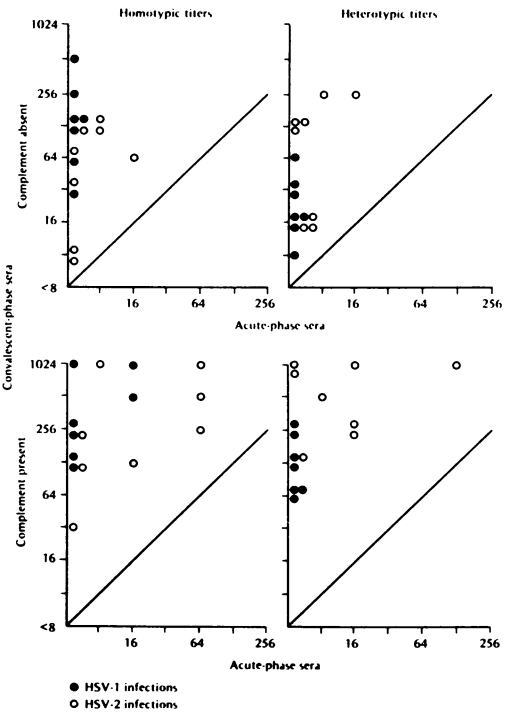


FIG. 1. *Comparison of acute and convalescent titers of homotypic and heterotypic HSV-neutralizing antibody in initial infections.*

Table 1 compares the degree to which complement enhanced homotypic and heterotypic neutralizing antibody in acute- and convalescent-phase serum specimens from patients with initial HSV infections and in sera from individuals with recurrent HSV infections. Titers of convalescent-phase sera, particularly from type 2 infections, tended to be enhanced by complement to a greater extent than those of acute-phase sera. Titers of sera from recurrent type 1 infections were not enhanced to as great a degree as were some of the sera from initial infections, but differences were not marked. Overall, there was no significant difference in the extent to which homotypic and heterotypic HSV antibody titers were enhanced by complement.

TABLE 1. *Complement enhancement of homotypic and heterotypic neutralizing antibody titers in initial and recurrent HSV infections*

| Patient group | Serum[a] | Test virus | Degree to which complement enhanced neutralizing antibody titers[b] | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 2X | 4X | 8X | 16X |
| HSV-1 initial infections (7 patients) | Acute | HSV-1 | 5[c] | | 2 | | |
| | | HSV-2 | 7[c] | | | | |
| | Conv. | HSV-1 | | 1 | 4 | 2 | |
| | | HSV-2 | | | 4 | 3 | |
| HSV-2 initial infections (8 patients) | Acute | HSV-1 | 3[c] | 2 | 2 | 1 | |
| | | HSV-2 | 3[c] | 1 | 2 | 2 | |
| | Conv. | HSV-1 | | | 3 | 3 | 2 |
| | | HSV-2 | | | 4 | 2 | 2 |
| Recurrent HSV-1 infections (6 patients) | | HSV-1 | | 2 | 4 | | |
| | | HSV-2 | | 4 | 2 | | |

[a] Acute-phase and convalescent-phase (conv.) sera.
[b] Values show number of sera.
[c] Titers <1:8 with and without complement.

**Complement enhancement of HSV-neutralizing antibodies in different classes of immunoglobulins.** Figure 2 (and also Table 2) compares the degree to which complement enhanced homotypic and heterotypic neutralization titers of IgM and IgG antibody in convalescent-phase sera from initial HSV infections. Homotypic IgG antibody generally showed at least as much enhancement by complement as did IgM antibody from the same serum. IgM neutralizing antibody to HSV-2 was not demonstrable in sera from HSV-1 infections (see also Table 2), but the patients produced heterotypic IgG antibody which was enhanced by complement. One patient infected with HSV-2 failed to produce heterotypic IgM antibody, but the other seven all produced heterotypic IgM neutralizing antibody which was enhanced by complement to the same or slightly lesser extent than heterotypic IgG antibody.

The low levels of neutralizing antibody present in acute-phase sera did not permit a valid comparison of the complement enhancement of IgM and IgG antibody in acute- and convalescent-phase sera.

**Specificity of the IgM neutralizing antibody response in HSV infections of humans.** Table 2 presents more detailed information on the levels of IgM and IgG HSV-neutralizing antibody in patients with initial and recurrent infections. First, it is seen that patients with initial HSV-1 infections produced fairly high levels of homotypic IgM antibody, but heterotypic antibody was not demonstrable in this immunoglobulin class, even in the presence of complement. On the other hand, all except one of the patients with initial HSV-2 infections produced heterotypic IgM neutralizing antibody to HSV-1 at levels comparable to or higher than those produced to homotypic HSV-2.

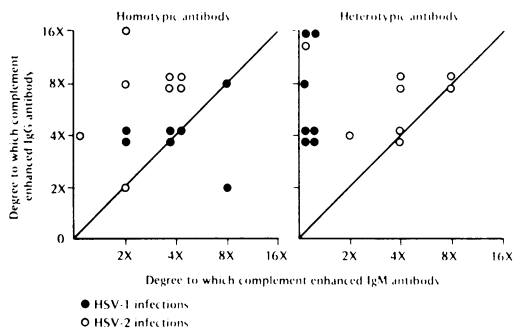


FIG. 2. *Comparison of complement enhancement of HSV-neutralizing antibody in IgM and IgG classes of immunoglobulins.*

Patient V. Co., a 9-year-old female with meningitis, showed an increase in HSV complement-fixing antibody from a titer of <1:8 to 1:128, and the infection was first considered to represent a probable primary HSV-1 infection. However, antibody typing by radioimmunoassay indicated HSV-1 antibody in both acute- and convalescent-phase serum specimens and a marked increase in HSV-2 antibody between acute and convalescent-phase serum specimens. The IgM antibody response was characteristic of an HSV-2 infection in that high levels of heterotypic IgM antibody were produced. Thus, the infection would appear to represent a current HSV-2 infection in an individual who had experienced a past infection with HSV-1. The results also indicate that an IgM antibody response can be elicited in a primary infection with a second HSV type. On the other hand, sera from three individuals with recurrent HSV-1 infections, and who currently had herpetic lesions, showed no IgM antibody.

TABLE 2. *IgM and IgG neutralizing antibody in HSV infections of humans*

| Patient category | Clinical syndrome | Patient | Age (years) | Sex | Serum (days after onset) | IgM antibody titer | | | | IgG antibody titer | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | HSV-1 | | HSV-2 | | HSV-1 | | HSV-2 | |
| | | | | | | −C[a] | +C | −C | +C | −C | +C | −C | +C |
| Initial HSV-1 infections | Dermatitis | J.Ca[b] | 9 | M | 28 | 16 | 32 | <8 | <8 | 256 | 1,024 | 32 | 256 |
| | Dermatitis | L.Ta[b] | 13 | F | 17 | 16 | 64 | <8 | <8 | 32 | 128 | 16 | 64 |
| | Stomatitis | K.Mo | 12 | M | 17 | 16 | 128 | <8 | <8 | 128 | 1,024 | 16 | 256 |
| | Stomatitis | B.Ba | 22 | M | 31 | 8 | 32 | <4 | <4 | 256 | 1,024 | 16 | 256 |
| | Stomatitis | B.Go | 27 | F | 20 | 8 | 16 | <8 | <8 | 32 | 128 | 8 | 64 |
| | Stomatitis | R.Fe[b] | 40 | F | 13 | 16 | 64 | <8 | <8 | 32 | 128 | 16 | 64 |
| | Meningitis | W.Ka[b] | 17 | M | 16 | 16 | 128 | <8 | <8 | 32 | 64 | 16 | 64 |
| Initial HSV-2 infections | Genital herpes | L.Wi[c] | 19 | F | 21 | 16 | 128 | 8 | 32 | 64 | 512 | 16 | 128 |
| | Genital herpes | S.Al | 20 | F | 25 | 16 | 64 | 8 | 32 | 64 | 512 | 128 | 1,024 |
| | Genital herpes | S.Ba[c] | 21 | F | 19 | <8 | <8 | 16 | 64 | 16 | 256 | 64 | 512 |
| | Genital herpes | F.Al | 22 | F | 21 | 16 | 64 | 8 | 32 | 32 | 256 | 32 | 256 |
| | Genital herpes | L.Ca[c] | 26 | F | 17 | 8 | 16 | 16 | 32 | 16 | 64 | 8 | 16 |
| | Meningitis | P.Pa. | 17 | F | 16 | 16 | 64 | <8 | 8 | 64 | 256 | 16 | 256 |
| | Meningitis | M.Ca | 22 | F | 17 | <8 | 32 | 8 | 16 | 16 | 128 | 8 | 64 |
| | Meningitis | J.Bl | 24 | F | 16 | 16 | 64 | 16 | 16 | 64 | 256 | 64 | 256 |
| Previous HSV-1, current HSV-2 infection | Meningitis | V.Co | 9 | F | 24 | 32 | 128 | 32 | 128 | 64 | 256 | 64 | 256 |
| Recurrent HSV-1 infections | Facial herpetic lesions | K.Du[b] | Adult | F | 7 | <8 | <8 | <8 | <8 | 128 | 512 | 64 | 128 |
| | | H.Ho | Adult | F | 7 | <8 | <8 | <8 | <8 | 256 | 1,024 | 8 | 32 |
| | | R.Ed | Adult | M | 6 | <8 | <8 | <8 | <8 | 256 | 512 | 16 | 32 |

[a] −C, Complement absent; +C, complement present.
[b] HSV-1 isolated.
[c] HSV-2 isolated.

## DISCUSSION

Infants with primary HSV infections were reported by Yoshino et al. (12, 13) to possess high levels of neutralizing antibody demonstrable only in the presence of complement in their early serum specimens, and it was suggested that the demonstration of complement-requiring neutralizing antibody might be used for diagnosis of primary HSV infections. However, in older patients and in recurrent infections, Yoshino et al. (12, 13), as well as others (6, 8), have generally found no more than fourfold enhancement of HSV-neutralizing antibody titers by complement. In the present study on initial HSV infections in children and adults, sera from one-half of the patients showed no more than fourfold enhancement of HSV-neutralizing antibody titers by complement, and sera from a high proportion of patients with recurrent HSV-1 infections, and without current herpetic lesions, showed the same degree of enhancement. These findings lend support to the concept that diagnosis of primary HSV infections can not be accurately accomplished simply by demonstration of complement-requiring neutralizing antibodies.

Previous studies on complement enhancement of HSV antibodies in human infections have apparently been performed using HSV-1 strains and predominately patients with ostensible HSV-1 infections (based on site of involvement). The present investigations on patients for whom the infecting virus type was clearly established by antibody typing, and in some cases by virus isolation as well, have shown that complement enhancement of antibodies elicited by HSV-2 is similar to that of antibodies produced by HSV-1 and, further, that homotypic and heterotypic HSV-neutralizing antibodies are enhanced to about the same degree. These findings are at variance with those reported by Lerner et al. (10) on rabbits hyperimmunized with HSV-1 and HSV-2. Animals immunized with HSV-1 produced significant levels of homotypic complement-requiring neutralizing antibody for the first 21 days after the beginning of immunization; heterotypic antibody, however, was produced at lower levels, was enhanced slightly by complement, and was not detectable until 3 to 4 weeks after the beginning of immunization. In rabbits immunized with HSV-2, production of homotypic and heterotypic neutralizing antibody was generally not detectable until after 21 days, and the antibodies showed little enhancement by complement.

However, the antigenic stimulus in immunized animals can not be considered comparable to that in natural infections of man.

As concerns complement dependence of HSV-neutralizing antibodies in different classes of immunoglobulins, our results with sera from human infections differed from those reported by Hampar et al. (5) on immune rabbits. The latter investigators found IgM neutralizing antibody to be enhanced to a greater extent than IgG antibody, but in our studies IgG antibody showed as much or more enhancement than IgM antibody from the same serum specimens. However, the times of collection of the human sera used in our study were not strictly comparable to those of the rabbit antisera. Also, species may vary in their complement requirements of HSV-neutralizing antibody in different classes of immunoglobulins.

In rabbits immunized with HSV-1 and HSV-2, Hampar et al. (4) found that late, complement-dependent 19*S* neutralizing antibodies (collected 2 weeks after a series of six weekly immunizations) possessed marked type specificity, whereas early 19*S* antibodies (collected 7 days after the beginning of immunization) did not. In the present studies on human sera, only IgM antibodies from HSV-1 infections showed type specificity.

Farris et al. (Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, p. 256, V 333) reported that antibody in 19*S* gradient fractions from HSV-2 patients reacted type specifically with HSV-2 antigens in IHA tests, whereas 19*S* antibody from HSV-1 patients showed cross-reactivity with type 1 and type 2 antigens. Also, in a study conducted in this laboratory (1), it was noted that a number of patients with HSV-2 infections had type-specific, mercaptoethanol-sensitive IHA antibodies in their acute-phase sera, but had no neutralizing antibody to either HSV type. The reason for differences in the reactivity of HSV IgM antibodies in neutralization and IHA test systems remains to be elucidated.

Using the indirect fluorescent antibody test for detection of virus-specific IgM and IgG antibody, Kurtz (7) demonstrated positive IgM staining reactions of HSV-1-infected cells with sera from HSV-2 infections. However, HSV-2-infected cells were not used in the study, so it is uncertain whether cross-reactivity of HSV IgM antibody is reciprocal in the fluorescent antibody test system or only one way as in the neutralization system.

Studies on additional patients will be needed to determine whether IgM neutralizing antibody responses are generally elicited in primary HSV infections superimposed upon a past infection with the viral heterotype, as was seen with a single patient in our study. However, Farris et al. (Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, p. 256, V 333) demonstrated an HSV-2 IgM antibody response by IHA in a similar case with a history of recurrent herpes labialis and HSV-1 antibody and a current, primary HSV genital infection.

## ACKNOWLEDGMENTS

We are indebted to Juanita Dennis for assistance is these studies.

This study was supported by Public Health Service research grant AI-01475 from the National Institute of Allergy and Infectious Diseases.

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